



Prevalence of *Escherichia coli* Carrying *pks* Islands in Bacteremia Patients

Eunyoung Lee, B.S. and Yangsoon Lee, M.D., Ph.D.

Department of Laboratory Medicine, Hanyang University College of Medicine, Seoul, Korea

Escherichia coli can harbor genomic *pks* islands that code for a polyketide-peptide genotoxin known as colibactin. *E. coli* strains carrying *pks* islands trigger genetic instability. *pks* islands have been significantly associated with bacteremia. We investigated the molecular epidemiology of bacteremic *E. coli* isolates and the prevalence of bacteremia-causing *E. coli* carrying *pks* islands. A total of 146 *E. coli* isolates were collected at a tertiary-care hospital from January 2015 to December 2016. The phylogenetic groups were determined by multiplex PCR. All isolates were screened by PCR for sequence type 131 (ST131)-associated single-nucleotide polymorphisms (SNPs) in *mdh* and *gyrB*. For detection of *pks* islands, we performed PCR for the *clbB* and *clbN* genes as colibactin system markers. Phylogenetic group B2 was the most common, accounting for 54.1% (N=79) of the isolates, followed by group D with 29.5% (N=43), group A with 11.6% (N=17), and group B1 with 4.8%. Of the group B2 isolates, 40.5% were ST131 strains and 32.9% carried *pks* islands. Only three ST131 isolates in group B2 carried the *clbB* and *clbN* genes, while the other 23 ST131 isolates did not. The *pks* gene might not be associated with ST131 strains.

Key Words: *Escherichia coli*, *pks* islands, *clbB*, Bacteremia, ST131

Escherichia coli is one of the most common bacteremia-causing pathogens; it has several virulence factors associated with bloodstream invasion and infection [1, 2]. Of these, the *papG* class II gene is thought to play a more important role in the development of *E. coli* bacteremia in patients with an upper urinary tract infection (UTI) than in patients with acute cholangitis [2]. However, the role of *E. coli* virulence factors in the pathogenesis of bloodstream infections remains unclear. Among its virulence factors, *E. coli* can harbor genomic *pks* islands that code for a polyketide-peptide genotoxin known as colibactin. *E. coli* strains carrying *pks* islands induce DNA damage and trigger genetic instability [3], and *pks* islands were significantly associated with bacteremia [4]. Therefore, we investigated the molecular epidemiology of bacteremic *E. coli* isolates and the prevalence of bacteremia-causing *E. coli* carrying *pks* islands in Korea.

A total of 146 *E. coli* isolates (one isolate per patient) were collected from blood samples consecutively at a tertiary-care hos-

pital from January 2015 to December 2016. Blood cultures were incubated in the BacT/ALERT system (bioMérieux, Marcy-l'Étoile, France). Each isolate was identified using the MicroScan Walkaway (Beckman Coulter, Brea, CA, USA) or Bruker Biotyper (Bruker Daltonics, Bremen, Germany) matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry systems. Antimicrobial susceptibility testing (AST) was performed using the MicroScan Walkaway system (Beckman Coulter). The AST results were interpreted based on the CLSI guidelines [5]. Phylogenetic groups were determined by multiplex PCR using a combination of three genes (*chuA*, *yjaA*, and *TSPE4.C2*), as previously described; the isolates clustered into four main phylogenetic groups, A, B1, B2, and D [6]. All isolates were screened by PCR for sequence type 131 (ST131)-associated single-nucleotide polymorphisms (SNPs) in *mdh* and *gyrB* [7]. For detection of *pks* islands, we performed PCR for the *clbB* and *clbN* genes as colibactin system markers, as previously described [4].

Received: May 22, 2017

Revision received: June 19, 2017

Accepted: December 28, 2017

Corresponding author: Yangsoon Lee
Department of Laboratory Medicine,
Hanyang University Seoul Hospital,
Hanyang University College of Medicine,
222-1 Wangsimni-ro, Seongdong-gu,
Seoul 04763, Korea
Tel: +82-2-2290-9655
Fax: +82-2-2290-9193
E-mail: yangsoon@hanyang.ac.kr

© Korean Society for Laboratory Medicine

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

The primary source of bacteremia was determined according to clinical presentation and/or evidence of an identical strain cultured near or on the same date as the onset of bacteremia. If the source of bacteremia could not be identified, it was classified as unknown origin.

Of the 146 bacteremic *E. coli* isolates, 107 (73.3%) were isolated from the bloodstream of patients with UTIs, and 13 (8.9%) were isolated from the bloodstream of patients with biliary tract infections (Table 1). An association with the source of infection was found for the resulting phylogenetic *E. coli* groups. Groups B2 and D were detected exclusively in UTIs (98/107, 91.6%), whereas group A was predominantly implicated in biliary tract infections (5/13, 38.5%). Twenty-eight isolates (group A=6, B2=14, and D=8) were recovered from patients with cancer.

Phylogenetic group B2 was the most common, accounting for 54.1% (N=79) of the isolates, followed by group D with 29.5% (N=43), group A with 11.6% (N=17), and group B1 with 4.8% (N=7; Fig. 1). Many strains belonging to group B2 are known to cause extraintestinal infections [8]. Phylogenetic analysis in a previous Korean study revealed that the majority of strains responsible for UTIs belonged to phylogenetic groups B2 (79%) and D (15%) [9]. Group B2 was also the dominant group causing bacteremia in the present study.

E. coli ST131 represents a recently emerging, globally disseminated cause of multidrug-resistant extraintestinal infections [10]. In this study, ST131 strains accounted for 24.7% (36/146) of all isolates tested. ST131 was found in groups B2 (N=32) and D (N=4), but not in groups A and B1. ST131 strains comprised 29.5% (36/122) of group B2 and D isolates.

Of the 146 bacterial isolates, 17.8% (26/146) carried *pk*s islands, all of which belonged to group B2. Only three ST131 strains carrying *pk*s islands were identified (2.1%). A number of studies

have reported that *pk*s islands are confined to group B2 strains [4, 8]. Similarly, in this study, the *clbB* and *clbN* genes were detected only in group B2 isolates. This suggests that *clbB* is an excellent marker for group B2. Johnson *et al* [4] have suggested that group B2 *E. coli* isolates carrying *pk*s islands increase the likelihood of bacteremia. They found that these isolates were present in 58% of blood samples, but only in 32% of fecal samples. In our study, 32.9% of the group B2 isolates from blood contained the *clbB* and *clbN* genes. Thus, the overall *pk*s prevalence was lower than that reported in the previous study (17.8% vs 58%) [4]. We found that only three ST131 isolates in group B2 carried the *clbB* and *clbN* genes, whereas the other 23 ST131

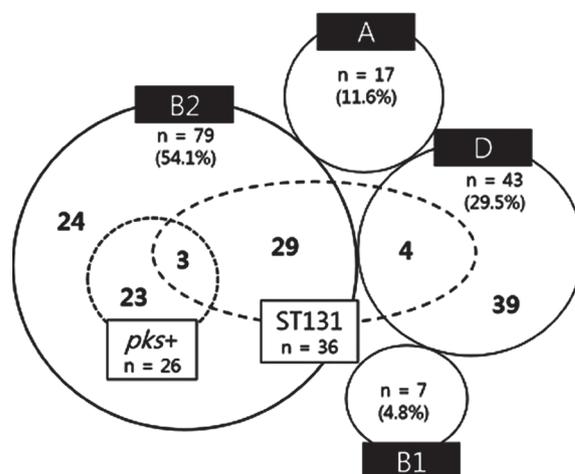


Fig. 1. Isolates carrying *pk*s islands and those identified as strain ST131, arranged by phylogenetic group. Phylogenetic groups were determined by multiplex PCR using a combination of three genes (*chuA*, *yjaA*, and *TSPE4.C2*), and the isolates were clustered into four main phylogenetic groups, A, B1, B2, and D. The numbers in the dotted circle and the small dotted circle indicate ST131 positive isolates and *pk*s-carrying isolates, respectively.

Table 1. Primary source of bacteremia classified according to phylogenetic group

Source of infection	Phylogenetic group				Total
	A	B1	B2	D	
Urinary tract	6 (5.6)*	3 (2.8)	67 (62.6)	31 (29.0)	107
Biliary tract	5 (38.5)	1 (7.7)	4 (30.8)	3 (23.1)	13
GI tract	1 (14.3)	0	3 (42.9)	3 (42.9)	7
Respiratory tract	0	0	4 (80.0)	1 (20.0)	5
Unknown	5 (20.8)	3 (12.5)	8 (33.3)	8 (33.3)	24
Total	17 (11.6)	7 (4.8)	79 (54.1)	43 (29.5)	146

Phylogenetic groups were determined by multiplex PCR using a combination of three genes (*chuA*, *yjaA*, and *TSPE4.C2*), and the isolates were clustered into four main phylogenetic groups, A, B1, B2, and D.

*Data are presented as number (%) of isolates.

Abbreviation: GI, gastrointestinal.

isolates did not. These results suggest that the *pks* gene might not be associated with ST131 strains.

Our results describe the molecular characteristics of *E. coli* isolated from bloodstream infections in a Korean hospital. Approximately 54.1% of bacteremic *E. coli* isolates belonged to phylogenetic group B2. Of the group B2 isolates, 40.5% were ST131 strains and 32.9% carried *pks* islands.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Acknowledgements

This study was supported by the research fund of Hanyang University (HY-201600000002782).

REFERENCES

1. Bonacorsi S, Houdouin V, Mariani-Kurkdjian P, Mahjoub-Messai F, Bingen E. Comparative prevalence of virulence factors in *Escherichia coli* causing urinary tract infection in male infants with and without bacteremia. *J Clin Microbiol* 2006;44:1156-8.
2. Wang MC, Tseng CC, Chen CY, Wu JJ, Huang JJ. The role of bacterial virulence and host factors in patients with *Escherichia coli* bacteremia who have acute cholangitis or upper urinary tract infection. *Clin Infect Dis* 2002;35:1161-6.
3. Cuevas-Ramos G, Petit CR, Marcq I, Boury M, Oswald E, Nougayrede JP. *Escherichia coli* induces DNA damage in vivo and triggers genomic instability in mammalian cells. *Proc Natl Acad Sci USA* 2010;107:11537-42.
4. Johnson JR, Johnston B, Kuskowski MA, Nougayrede JP, Oswald E. Molecular epidemiology and phylogenetic distribution of the *Escherichia coli* *pks* genomic island. *J Clin Microbiol* 2008;46:3906-11.
5. CLSI. Performance standards for antimicrobial susceptibility testing. 25th ed. CLSI document M100-S25. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
6. Doumith M, Day MJ, Hope R, Wain J, Woodford N. Improved multiplex PCR strategy for rapid assignment of the four major *Escherichia coli* phylogenetic groups. *J Clin Microbiol* 2012;50:3108-10.
7. Johnson JR, Menard M, Johnston B, Kuskowski MA, Nichol K, Zhanel GG. Epidemic clonal groups of *Escherichia coli* as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002 to 2004. *Antimicrob Agents Chemother* 2009;53:2733-9.
8. Nougayrede JP, Homburg S, Taieb F, Boury M, Brzuszkiewicz E, Gottschalk G, et al. *Escherichia coli* induces DNA double-strand breaks in eukaryotic cells. *Science* 2006;313:848-51.
9. Lee JH, Subhadra B, Son YJ, Kim DH, Park HS, Kim JM, et al. Phylogenetic group distributions, virulence factors and antimicrobial resistance properties of uropathogenic *Escherichia coli* strains isolated from patients with urinary tract infections in South Korea. *Lett Appl Microbiol* 2016;62:84-90.
10. Nicolas-Chanoine MH, Bertrand X, Madec JY. *Escherichia coli* ST131, an intriguing clonal group. *Clin Microbiol Rev* 2014;27:543-74.